Crossing biological membranes

Professor Jeffrey Green is one of the Directors of a UK research network dedicated to solving one of the most under-researched industrial biotechnology problems – how substances cross biological membranes. He explains how the Crossing Biological Membranes Network encourages crossdisciplinary collaborations to ensure progress in the field.

Can you explain the impetus behind the Crossing Biological Membranes Network (CBMNet)? What are its main aims in terms of supporting research in industrial biotechnology and bioenergy?

CBMNet is centred on the premise that the transport of solutes and proteins across biological membranes is a fundamental biological process that plays a crucial, but understudied, role in almost all cell factory-based industrial biotechnology applications.

How does CBMNet fit within the broader aims of BBSRC NIBB and what makes your Network particularly unique?

Our Network is classified as one of three cross-cutting NIBB, because its outputs have such broad implications and will impact on almost all cell factory-based industrial biotechnology processes. Our role in the NIBB landscape is to act as advocates for optimising biological membrane function in the design of new bacterial, yeast and mammalian cell biocatalysts.

Amongst the many strengths of CBMNet are its cross-cutting nature and focus on a pool of academic expertise that has not yet been widely accessed by the industrial biotechnology sector. Thus, we are uniquely placed to be outward facing and interact synergistically with other NIBB and beyond.

In what ways has the Network facilitated collaboration between its members, particularly between industrial and academic researchers?

Our events have been the major route to foster new and existing collaborations and have also led to project funding. To date we have provided over £175,000 of funding for innovative Proof of Concept projects and Vacation Scholarships bridging academia and industry. We have also funded five Business Interaction Vouchers worth over £30,000 and helped over 30 academics from 20 institutions engage with more than 15 companies to secure funding. We have many more ideas for exciting workshops, training courses, events and public engagement activities for the future, so watch this space!

Open cooperative partnerships are often essential for making the transformative steps needed for progress and can be the most rewarding and exciting way to do science. This is where NIBB can make a difference – facilitating academic-industry partnerships for carrying out vital precompetitive research that will benefit everyone.

How has industry been involved with the Network?

We currently have over 100 industry members, making up approximately 20 per cent of our membership. We are actively engaged, and have directly funded projects, with Lucite, Green Biologics Ltd, Croda, Johnson Matthey, TeGene Ltd, Unilever, FujiFilm Diosynth Biotechnologies and the Centre of Process Innovation. Guided by input from commercial practitioners, we hope to create innovative solutions to real industrial biotechnology problems. Furthermore, we aim to translate novel biological knowledge into bio-industrial know-how and new opportunities, based on improved process efficiencies.

In your opinion, what has been the Network’s biggest success so far?

One of our greatest achievements has been a Proof of Concept project that provided essential data for a BBSRC LINK grant worth £421,000. The project, entitled ‘Bacteria transport and catabolism of human malodour precursors’, is a collaboration between CBMNet Co-Director Dr Gavin Thomas, University of York, and Unilever.

In addition, data from one of our Business Interaction Vouchers have been used to secure a BBSRC Industrial Biotechnology Catalyst grant worth £3.8 million. This project will investigate productive whole cell biocatalysis by engineering resistance to toxic products and substrates.

From a personal viewpoint, the greatest success has been the creativity and enthusiasm of the CBMNet Management Board and wider membership. Participating in many lively discussions and learning more about the needs of industrial biotechnology practitioners has been incredibly rewarding.
A commitment to long-term impact on industry

The Crossing Biological Membranes Network settled on seven themes on which to focus its funding and networking. These evolved from initial discussions with Network members and aim to positively affect existing and developing biotechnology processes.

GETTING MORE THINGS IN: MANIPULATION OF SUBSTRATE UPTAKE

The first step in any cell factory-based process must be the transport of reactants across the lipid membrane and into the cell. For example, during the production of amino acids for food products, engineered forms of transporters have been used to optimise the uptake of the hydantoin feedstock.

The Network aims to use genetic resources and molecular genetics to expand the industry’s repertoire of substrate transport options and help optimise the kickstarting of a cell factory-based process. This could also enable the use of feedstocks that could not have previously been used.

GETTING THINGS OUT: IMPROVING EXPORT/ EFFLUX OF CHEMICALS

Many industrially useful chemicals are highly toxic. Accumulation of these products to greater than millimolar concentrations will kill the cell.

CBMNet members will investigate new applications for natural or re-engineered efflux or export systems, exploiting the specialised membrane channels that nature has cultivated to keep the live reactor healthy. This could include using the widely studied AcrB, TolC and EmrAB transporters to expel free fatty acids from Escherichia coli for biodiesel synthesis.

HIJACKING TRANSPORTERS FOR INDUSTRIAL BIOTECHNOLOGY AND BIOENERGY

Fundamental knowledge of the structure/function relationships of transporters can be exploited for biotechnology, because the transporters have relaxed substrate specificity and thus will recognise and act on modified substrates. For example, researchers in the US have evolved the E. coli efflux pump AcrB to excrete toxic short-chain alcohols, thereby improving tolerance.

MOVING COMPLEX MOLECULES ACROSS MEMBRANES

Secreting and post-translationally modifying complex molecules such as proteins within cell factories – including yeast and Chinese hamster ovary (CHO) cells – can involve passage across several biological membranes, which can lead to significant bottlenecks for high productivity. By leveraging molecular methods and engineering expertise, the Network is seeking to increase the yield of non-native proteins from cells.

ALTERING THE MEMBRANE ITSELF

The structure and functions of biological membranes are defined by their protein and lipid composition.

CBMNet researchers aim to better understand how cell membranes can be engineered in cell factory systems for heightened resistance to stress and toxicity – improving the lifetime of the cell factory – and efficient transport of reactants and products. One aim of the Network will be to functionalise the membrane for release of hydrocarbons, as build-up of long chain hydrocarbons intended for transportation fuel is a common challenge.

PUTTING IT ALL TOGETHER: CONSOLIDATED BIOPROCESSING

Combining all of the above themes can really leverage the knowledge of the Network for improved yield and process efficiency for industrial biotechnology. “One might wish to consider how to maximise substrate generation by optimising secretion of degradative enzymes; identify suitable transport systems for substrate uptake; determine whether the properties of selected transport systems are fit for industrial biotechnology purposes [integrating these new genes into regulatory circuits for optimum expression]; maximise product recovery by efficient excretion; and minimise product toxicity by modifying membrane properties,” Professor Jeffrey Green sums up.

With the synthetic biology and metabolic modelling expertise of the Network’s researchers, CBMNet is perfectly positioned to do this.

SOCIOECONOMIC CHALLENGES RELATED TO CROSSING BIOLOGICAL MEMBRANES

To ensure that CBMNet’s work has lasting effects on the biotechnology sector and beyond, it also works with groups such as the Sheffield Political Economy Research Institute (SPERI) and Science and Technology in Society (SATIS) group.

SPERI is studying the new markets created by biotechnology opportunities and how these will impact the economy. SATIS will complement this by considering science and society relations, with a team that focuses on synthetic biology, process engineering and biosciences.

CBMNet will also host a joint event with another of the Networks in Industrial Biotechnology and Bioenergy to consider the societal issues raised by the application of new technologies in the industrial biotechnology sector in the future.

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**Synthesising new biological research networks**

Although the UK biotechnology industry is going from strength to strength, many companies are missing an important piece to their bioprocessing puzzles. The **Crossing Biological Membranes Network** is striving to close the industry’s knowledge gaps concerning the impacts of biological membrane function in biotechnology.

**GONE ARE THE** days when giant factories were belting out smoke and pollutants. With climate change becoming an ever-growing concern, researchers and industrialists are typically looking to a cleaner future where inputs and outputs – substrates and products – are processed in a greener fashion.

Nature has been improving its own chemical processes for millions of years, and many of these are carried out at ambient temperatures and pressures – a far cry from the extreme heat and pressure used in traditional industrial processes. Synthetic biology aims to make use of these highly refined biological organisms, reactions and components, and re-engineer them for defined purposes. This increasingly prominent field could make some industrial processes significantly cleaner, as well as expand the repertoire of useful chemicals that we can produce, including new fuels, pharmaceuticals and biopharmaceuticals.

Many synthetic biology approaches for industrial biotechnology rely on a cell factory. This factory starts with a chassis organism – for example *Escherichia coli*, a well-studied and tractable bacterial species – with many non-essential genes removed. The chassis provides a blank page for an engineer to ‘rewire’ the cell’s metabolic networks for synthesis of the desired chemical from supplied feedstocks. While such engineering processes are being continuously refined, stumbling blocks are often encountered when scaling up these cell factories to a commercially viable industrial process.

**BRIDGING THE MEMBRANE BARRIER**

Professor Jeffrey Green of the University of York, and colleagues across the UK in industry and academia, recognised that maximising the yield of a desirable product is highly dependent on the function of biological membranes. Together, Green and Thomas applied for Biotechnology and Biological Sciences Research Council (BBSRC) funding to establish the Crossing Biological Membranes Network (CBMNet), one of 13 UK-based BBSRC Networks in Industrial Biotechnology and Bioenergy (NIBB).

"Any cell factory-based process will be affected by biological membrane function," explains Green, now one of the Directors of CBMNet. "For example, transporting substrates into the factory, trafficking intermediates between organelles within the factory and exporting products from the factory all involve crossing biological membranes, and each of these is a potential bottleneck that lowers process efficiency."

CBMNet’s impact is already evident, despite being only one year into its programme.

Bringing together engineers, chemists and biologists, the Network established a five-year programme in July 2014. Within this, it aims to deliver events and funding for growing partnerships between academia and industry that focus on overcoming barriers presented by the membrane. The challenge the Network set itself was to increase the understanding of cell transport mechanisms and develop process solutions for specific industrial biotechnology problems.

**SUPPORTING TRANSLATIONAL RESEARCH**

One of CBMNet’s grandest aims is the realisation of years of academic membrane
research as tangible applications in the biotechnology industry. To this end, it acts as a ‘matchmaking’ service; thus many of its events serve as opportunities for industry and academic scientists to meet and discuss ideas.

This goal is best embodied by the Network’s Industry Partnering Workshops. At these events, teams of scientists are formed to tackle genuine industrial problems, with help given to ready multidisciplinary groups to apply for CBMNet Proof of Concept funds and other grants. Mini sessions are also offered to raise ideas and problems with experimental methodology and techniques, enabling productive knowledge exchange.

EXPANDING HORIZONS

CBMNet’s impact is already evident, despite being only one year into its programme. So far it has funded seven Proof of Concept grants along with seven Vacation Scholarships (worth over £175,000) and five Business Interaction Vouchers. Many of these awards have focused on supporting students and early career researchers to ensure that the biotechnology expertise continues to grow.

“In our first year we have begun to establish an active and engaged community of industrialists and academics,” Green emphasises. “We now need to build on these foundations and promote the importance of an appreciation of the impact that membrane biology can have on industrial biotechnology processes through our project funding streams and meetings.”

Most importantly, CBMNet is looking to further strengthen its links with industry so that the success of the UK membrane research community is translated into practice. As the Network continues to expand, the sum of its collective knowledge will be of immense use to the biotechnology industry as a whole.

CHANGING THE FACE OF UK BIOTECHNOLOGY, ONE PROJECT AT A TIME

THE BEAUTY OF N-BUTANOL

Green Biologics Ltd uses solventogenic Clostridia to produce renewable n-butanol, a chemical used in paint and adhesive manufacture. However, n-butanol is costly to purify from the bacterial fermentation mixture. n-Butanol is toxic to the bacteria, and Green Biologics wanted to find out why.

With a CBMNet Business Interaction Voucher, the company worked with University of Lincoln researcher Dr Alan Goddard to understand how n-butanol affects the bacterial lipid membrane. Goddard discovered that the chemical was indeed disrupting the solventogenic Clostridia membranes. Using further CBMNet funding, Green Biologics is now attempting to evaluate the protective mechanisms of the cell when it experiences stress from high levels of n-butanol.

BOOSTING BIOSURFACTANT PRODUCTION

Another biotechnology company, Croda, sought the help of CBMNet’s Business Interaction Vouchers to investigate the intricacies of producing biosurfactants – substances of immense commercial value. Its desired product was mannosylerythritol lipids (MEL), but the production process was suffering from limited yields from the organism that synthesises them, Pseudomonas aphidis.

Professor Douglas Kell CBE of the Manchester Institute of Biotechnology, in cooperation with a University of Manchester bioinformatician, Dr Ryan Ames, analysed the P. aphidis genome to identify possible MEL transporters. The academic researchers found candidate transporters, and the collaboration with Croda is expected to continue to develop metabolic network models to, in theory, optimise the biosurfactant production process.

“CBMNet has turned out to be one of the most exciting networks – opening up a whole new landscape with rich potential for addressing issues of which we have been blissfully ignorant,” Doug Cossar of Croda commented after partnering with Kell and Ames.

CROSSING BIOLOGICAL MEMBRANES NETWORK

OBJECTIVES

- To foster collaborations between academia and industry dedicated to tackling challenges in industrial biotechnology and bioenergy
- To overcome yield restrictions due to difficulties with transporting substances into and out of cell factories
- To harness biological resources for producing and processing materials, biopharmaceuticals and energy

KEY COLLABORATORS

UK: Green Biologics Ltd • Unilever • Ingenza • ReBio Technologies Limited • Fujifilm Diosynth Biotechnologies • Lucite • Croda • The Centre for Process Innovation

FUNDING

Biotechnology and Biological Sciences Research Council (BBSRC)

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PROFESSOR JEFFREY GREEN

has over 25 years’ experience in the fields of microbial biochemistry and physiology and has published more than 100 papers and reviews. At present, he is a Professor in the Department of Molecular Biology and Biotechnology at the University of Sheffield and additionally has a position on the management board of the White Rose BBSRC Doctoral Training Partnership and the editorial board of Biotechnology Letters.

DR GAVIN THOMAS

has over 15 years of experience studying the function of microbial transport proteins and has published over 50 papers and reviews. His research focuses on the application of transporters to industrial biotechnology. He has an international reputation in bacterial transporter research, having made major advances in studying the function and mechanism of tripartite ATP-independent periplasmic (TRAP) transporters.

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